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TITLE: MELANOMA PRONE FAMILIES: NEW EVIDENCE OF DISTINCTIVE CLINICAL AND HISTOLOGICAL FEATURES OF MELANOMAS IN *CDKN2A* MUTATION CARRIERS

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ABSTRACT

Germline mutations on the *CDKN2A* gene, the most important known genetic factors associated with cutaneous melanomas (CMs), predispose carriers to multiple primary CMs (MPMs) with higher frequency and younger onset compared to non-carriers. Most of the largest published studies concerning clinical and histological characteristics of CMs with *CDKN2A* mutation carriers did not specify if the described CMs are first or subsequent to the first, and they used sporadic CMs from non-genotyped patients as controls.

We conducted a single-centre observational study to compare clinical and histological CM features of 32 unrelated carriers (MUT) of 5 germline *CDKN2A* mutations (one of which was never previously described) compared to 100 genotyped wild-type (WT) patients. We stratified the data based on time of diagnosis, anatomical site and histological subtype of CMs, demonstrating several significant unreported differences between the two groups. MUT developed a higher number of dysplastic nevi and MPMs. We proved for the first time that anatomical distribution of CMs in MUT was independent of gender, unlike WTs. MUTs developed *in situ* and superficial spreading melanomas (SSMs) more frequently, with significantly higher number of SSMs on the head/neck. In MUTs, Breslow thickness was significantly lower for all invasive CMs. When CMs were stratified on the basis of the time of occurrence, statistical significance was maintained only for SSMs subsequent to the first. In WTs, Clark level was significantly higher, and ulceration was more prevalent than in MUTs. Significant differences in ulceration were observed only in SSMs. In nodular CMs, we did not find differences in terms of Breslow thickness or ulceration between WTs and MUTs.

In situ CMs developed 10 years earlier in MUTs with respect to WTs, whereas no significant differences were observed about invasive CMs. In contrast to those reported previously by other authors, we did not find a difference in skin phototype.

Keywords: *CDKN2A*, familial melanoma, cutaneous melanoma, melanoma susceptibility genes, risk factor for cutaneous melanoma

Abbreviations: CM: Cutaneous Melanoma; PC: Pancreatic Cancer; MUT: carrier of *CDKN2A* germline mutation; WT: Wild-Type; UM: Uveal Melanoma; FCS: Familial Cancer Syndrome; N_CM1: First CM; N_CM1.5: CM diagnosed within 3 months after the first (Metachonous CM); MPMs: Multiple primary melanomas; SLNB: sentinel node biopsy; y: years; SNM: sentinel node metastases.

INTRODUCTION

To date, the major risk factor identified for cutaneous melanoma (CM) is a positive family history of this malignancy, which is reported in 5-15% of all melanoma patients [7,34,35]. Familial melanoma represents a genetically heterogeneous cancer, and several susceptibility genes during the last two decades have been identified. Autosomal dominant inherited germline mutations in the high-risk susceptibility genes, *CDKN2A* and, less frequently, *CDK4*, are the most important genetic factors associated with CM. Mutations in these genes are associated with an increased risk for both melanoma and pancreatic cancer (PC), recognized as "Melanoma-Pancreatic Cancer Syndrome" [24,34,35]. Pathogenic variants of *CDKN2A* have also been associated with an increased risk of other non-melanoma and non-PC tumours (overall 75% of the carriers at age 80 years): gastrointestinal (upper tract) and respiratory malignancies, childhood cancers (nephroblastoma and acute lymphoblastic leukaemia), squamous cancers of the head and neck and central nervous system tumours (recognized as "Melanoma and Neural System Tumour Syndrome") [24,34,35]. In *CDKN2A* carriers (MUT), cancers also appear to be strongly influenced by environmental risk factors. CM risk has been positively associated with sun exposure, whereas pancreas, respiratory or upper digestive neoplasms are up to 9 times more frequent in ever-smoking carriers compared with never-smoking carriers [21].

Several studies, summarized in Table 1, reported clinical and histological features of CMs in *CDKN2A* mutation carriers, which differed significantly from melanomas of non-pathogenic *CDKN2A* variant carriers. Nonetheless, limited data are available about certain clinical and histological characteristics of skin tumours of *CDKN2A* mutation carriers. Particularly, most of these studies have not specified if the compared CMs are first (index cases) or subsequent (incident cases) cases, and they have ~~are~~ used as controls sporadic CMs developed by non-genotyped patients. This finding may lead to bias since most melanoma-prone families are, unlike most sporadic cases, under close dermatologic surveillance with the aim to ~~of~~ diagnose tumours at earlier or premalignant stages [2,25,31,36,39].

Moreover, germline mutations in additional susceptibility genes that confer a genetic risk for familial CM and non-cutaneous tumours have been identified. *BAP1* acts as a tumour suppressor gene, and its germline mutations were reported to predispose patients to uveal (UM) and cutaneous melanoma, mesothelioma, renal cancer (RC), PC and basal cell carcinoma [1,5,10,12,40]. *MITF* confers an

increased risk for CM, RC, and PC; promoter of *TERT* and *POT1* demonstrated co-segregation with CM-prone families [8,19,22,23, 28,29,32].

We conducted a single-centre observational study to identify carriers of germline mutations in melanoma susceptibility genes (*CDKN2A, CDK4, MITF,* promoter of *TERT*, *BAP1* and *POT1*) in Caucasian patients with a history of CM undergoing periodic visits at our Department.

The main objectives of this study were to outline the clinical and histological characteristics of CMs developed by carriers of these mutations, investigating the missing clinical and histological data. The more precise characterization of the hereditary oncologic phenotype of these melanoma-prone families could allow a better definition of a specific dermatological surveillance programme for mutation carriers and their family members.

MATERIALS AND METHODS

Enrolment of Cutaneous Melanoma cases

We enrolled 300 histologically confirmed CM patients who have undergone dermatologic surveillance at our Institution. In detail, the follow-up schedule was based on AJCC staging according to the guidelines developed by the major Italian referral centres for melanoma, and it was applied regardless of the mutational status [29]. For AJCC stage I, the programme consisted of a total-body physical examination every 6 months for the first 5 years and thereafter yearly with no radiological procedures. For AJCC stage II, clinical examination was performed every 4 months in the first 5 years, then every 6 months from the 5th to the 10th year, and yearly after 10 years from the first diagnosis. For stage IB, an annual chest radiograph and abdomen ultrasound was performed for the first 5 years, whereas stage II patients underwent a computed tomography scan (TC). For stage III patients, imaging with FDG-PET and/or HR-TC scan was performed every 6 months for the first 5 years and then yearly [14,18].

All patients were interviewed about their personal and family history of CM and non-CM tumours. Specifically, patients who had at least one of the inclusion criteria (Table 2) that suggests a FCS (Familial Cancer Syndrome) were considered eligible for genetic testing.

This study obtained approval by the Ethics Committee of our Institution (Comitato Etico Interaziendale, Azienda Ospedaliero Universitaria Maggiore della Carità di Novara, Italy). Written informed consent for study participation was obtained from all patients.

Data Collection

We included 45% of 300 CM patients, which represented a sub-population of 134 non-related subjects at high risk not only for CM but also for other visceral cancers (high-risk patients). These patients were invited to participate in this study and were informed about its aims and limits. After providing written informed consent, peripheral blood for mutation analysis was obtained from all 134 high-risk patients.

All CM patients were Caucasian (131 of Italian descent, 1 Albanian, 1 Romanian and 1 Czech) and living in Italy. Clinical and histological cancer features, tumour site, disease stage, and age at diagnosis of CM was obtained from medical records, and a review of pathologic material and pathology reports was conducted for all CM patients. Familial recurrence of CM was checked using a questionnaire to interview patients about their first-, second-, and third-degree relatives.

Genotyping of candidate genes

We collected and processed blood samples as previously described in detail [10]. Genomic DNA was extracted using QIAamp® DNA Blood Maxi Kit (QIAGEN, Valencia, CA, USA) according to the manufacturer's protocol. Primers appropriately designed using the reference sequences provided by NCBI or Ensembl databases were used to amplify the exons and intron–exon boundaries of CDKN2A (NM_000077.4), the exon 2 of CDK4 (NM_000075.3), the promoter of TERT (NM_198253.2), the missense variant p.Glu318Lys of MITF (NM_000248.3), the 17 exons, intron–exon boundaries and promoter region (~1000 bp upstream of the ATG) of BAP1 (NM_004656.2) and exon 10 of POT1 (NM_015450). PCR reactions were performed in a 25 μL tube using GoTaq® Flexi Polymerase (PROMEGA, Madison, WI, USA) for BAP1 fragment amplification and a Taq Gold 360 + GC enhancer for fragment amplification of melanoma predisposition genes.

Statistical analysis

Statistical analysis was performed to evaluate clinical and histological differences in CMs between carriers and non-carriers of germline mutations in melanoma-susceptibility genes.

The distribution of continuous variables was assessed with Q-Q plots. Mean and standard deviation (SD) were estimated for continuous variables providing visual impression of normality. For these variables, the difference between study groups was assessed using Student's t-test. Median and interquartile range (IQR) were estimated for continuous variables that did not provide visual impressions of normality. For these variables, the difference between study groups was assessed using the Wilcoxon Mann-Whitney U-test. Differences in the distributions of categorical variables between study groups were assessed using Fisher's exact tests. All reported p-values are two-sided.

RESULTS

Mutational Analysis

Among the 300 histologically confirmed CM patients who have undergone dermatologic surveillance at our Department between 2011 to 2016, 134 have been genotyped because they met at least one of the inclusion criteria (Table 2) suggesting a FCS.

We identified 32 unrelated patients that were carriers of 5 different germline mutations (1 of which is a novel mutation) on the *CDKN2A* gene, and 1 patient (NFV20.1) was a carrier of a missense variant of the *CDKN2A* gene that has never been previously described in melanoma (Online Resource1); we also identified a patient with a unique *BAP1* deleterious variant previously published (NFp101.1) [10].

None of 134 patients were positive for any pathogenic germline variants on *CDK4*, *MITF*, the promoter of *TERT* or *POT1*.

The overall frequency of *CDKN2A* gene germline mutations in our series was 11%, accounting for 25% (33/133) of the selected high-risk patients.

Personal and Family Features of *CDKN2A* carriers

To investigate the distinctive clinical and histological features of CM from carriers (MUT) versus non-carriers (WT) of germline *CDKN2A* mutations, we compared the available data from the 32 MUT (21 F 65%, 11 M 35%) versus the 100 WT patients (67 F, 33 M). We excluded patients NFV20.1 and NFp101.1 from the statistical analysis due to their unique genetic and clinical features (Online Resource 1). Therefore, we analysed 132 CM patients (88 F 66.7%, 44 M 33.4%) affected by 179 CMs. Skin phototype, family history, clinical and histological data of CMs, and melanocytic and atypical nevi are summarized in Tables from 3 to 6.

Gender

The percentage of women (F) was significantly higher both among MUTs (65.7% P=0.05) and WTs (67%, P<0.001). Moreover, women developed most CMs regardless of genotype (71.7%, P=0.005 and 68%, P<0.001, respectively, in MUT and WT groups).

Phototype

We did not find significant differences between the two genotyped groups (P=0.759). According to the phenotypic characteristics most represented in our geographical area, most patients had Fitzpatrick skin phototype III, which was observed in 59.4% of MUTs and 65% of WTs, respectively, while 25% of MUTs and 19% of WTs presented with Fitzpatrick skin phototype II.

Dysplastic nevi and common melanocytic nevi

There was no difference in the number of total common melanocytic nevi (P=0.131). However, a personal history of previously excised dysplastic nevi was more frequent in MUT patients (62.5%); the comparison to the WT group (26%) showed a statistically significant difference (P<0.001).

Family History of CM and PC

A positive family history of CM and/or PC was detected in 90.6% (29/32) of MUTs and in 37% (37/100) of WTs (P<0.001). There was a statistically significant difference between the two genotyped groups in term of CM family history (MUT 78.1% vs WT 29%, P<0.001) and PC family history (MUT 34.4% vs WT 10%, P<0.001). Forty-one percent (54/132) of all patients included in this study had at least one relative with a history of CM. Among them, 46.3% (25/54) carried a *CDKN2A* germline mutation. Sixteen percent (21/132) of patients had ~~almost~~ at least one family member with a history of PC. Among them, 52.4% (11/21) carried a *CDKN2A* germline mutation.

Clinical Tumour Features of *CDKN2A* carriers

Age at diagnosis of CM

Considering both overall CMs and the first CM alone (N_CM1), we did not find a statistically significant difference between MUT and WT patients in the median age at diagnosis. However, the median age at diagnosis of *in situ* CM was approximately 10 years lower in MUTs than in WTs (Table 3). All CMs diagnosed before the age of 20 occurred among MUT patients. The percentage of CMs diagnosed within 40 years was 60.5% in MUTs and 45% in WTs.

Moreover, MUTs developed metachronous CMs (e.g., diagnosed within 3 months from the first one N_CM1.5) at a lower median age (29 y) compared to WTs (43 y; p=0.053) (Table 3).

Multiple primary melanomas (MPMs)

MUTs developed a statistically significant higher number of MPMs compared to WTs (Table 3). Although in both groups most of the patients developed 2 primary melanomas (61% among MUT, 87.5% among WT), 3 MPMs were observed more frequently in MUT than in WT subjects (28% vs 12.5%), and 4 MPMs were observed in 11% of MUT patients and in none of the WTs. The time interval between first and subsequent to the first melanoma was longer in MUT than in WT subjects. In detail, 27.7% (5/18) of MUTs developed N_CM2 after $\geq$ 5 y from the first one compared to 18.75% (3/16) of WTs (P=0.993).

Anatomical Site of CMs

Overall, the anatomical site most frequently involved in CMs was the trunk, followed by lower limbs, upper limbs, head, and neck region and acral sites without statistically significant differences between the two genotyped groups (MUT vs WT, P=0.288). Regarding the MUT group, the pattern of skin involvement was similar in F and M subjects (P=0.685). Contrarily, in WT subjects, the involved body sites were significantly different by gender (P=0.008; Table 3 and 4).

Concerning MPMs, most MUT patients (77.8%) developed multiple CMs at different anatomical sites, whereas in the WT population, only 47% of patients showed discordant body sites (P=0.057).

Histological Tumour Features of *CDKN2A* carriers

Histopathological features of CMs are summarized in Table 5 and 6 according to *CDKN2A* gene status.

Histological subtype

Overall, most CMs were SSMs (85%) followed by NMs (11%) and then by the other subtypes (4%), without statistically significant differences (P=0.512). As can be observed in Table 6, among invasive CMs, the percentage of SSMs was higher in MUT than in WT patients (92% vs 83%, P=0.156), whereas NMs were more represented in WTs (15% vs 6%, P=0.086).

Considering only the first invasive tumours, SSM was the more frequently observed histotype (88.5% of MUT and 81% of WT patients, P=0.382). On the other hand, NMs were more represented in the non-carrier group (11.5% in MUT vs 16.5% in WT subjects; P=0.545).

Globally, the frequency of *in situ* CMs was similar in the 2 groups (13.8% vs 14.9% of all CMs in MUT and WT). For only the first CMs, *in situ* CMs were more frequent in MUT patients (13.4% MUT vs 11.2% WT; P=0.758), and they were diagnosed at an earlier median age (34.5 y) compared to WTs (43 y). For subsequent CMs, we did not detect NMs; WTs developed *in situ* tumours more frequently than MUTs (P=0.068), who most commonly developed SSMs (P=0.061).

Stratifying histological data based on the anatomical site, we observed differences in the distribution of CMs for the head and neck region: all MUT patients developed SSMs, while WT patients generated mostly NMs or LM-LMMs (P=0.006). For the other anatomic sites, we did not observe statistically significant differences between the two groups. However, on lower limbs, 93.7% of MUT patients developed SSMs or *in situ* CMs (vs 76.3% of WT patients), while the 86% of NMs were found in WT patients.

Analysing MPMs, we noted that all patients, regardless of mutational status, tend to develop CMs with the same histological type (concordance of subtype of MPMs 63.2% MUT vs 70.8% WT P=0.594).

Breslow thickness and Clark level

MUT patients developed invasive CMs with significantly lower Breslow thickness than WTs. The results were significant both for all invasive CMs (median 0.4 mm vs 0.57 mm, P=0.023) and considering only the invasive CMs (all SSMs) after the first (mean 0.37 mm vs 0.73 mm, P=0.015). Among all invasive CMs, the percentage of lesions thicker ≥ than 1 mm was 12.2% in MUTs and 32% in WT patients. Contrarily, as regards only first invasive CMs, carriers compared to non-carriers developed skin tumours with higher Breslow thickness, both for all tumours regardless of subtype (1.04 mm vs 0.99 mm P=0.808) and for SSMs (0.96 mm vs 0.84 mm P=0.663). As regards NMs, we did not observe differences between MUT and WT patients (P=0.926).

Regarding the Clark level, the difference between MUT and WT patients was statistically significant for all invasive CMs (P=0.002), for first invasive CMs (P=0.029) and for the invasive CMs subsequent to the first (P=0.005). In all, 69.4% of CMs from MUT subjects were Clark level II compared to 39.2% of CMs from WT patients. In contrast, only 2% of CMs in MUT patients had a Clark level of IV, compared to 20.6% in the WT group.

Ulceration

Considering all invasive CMs, a histologically confirmed ulceration was found in 1.8% of MUTs and 11.9% of WTs (P=0.028). In contrast, considering only the SSMs, ulceration was presented exclusively in CMs from WT patients (P=0.036).

For all NMs, we did not observe differences between MUT and WT patients.

Mitotic rate

Mitotic rate (number of mitoses/mm^2) was evaluable for 55.5% (86/179) of CMs, all diagnosed between 2009 and 2016 when this histological feature was introduced as a prognostic factor in the seventh edition AJCC Staging System [4]. We did not observe a statistically significant difference between the two genotyped groups: 74% vs 67% of all invasive CMs developed by MUT and WT patients, respectively, had less than 1 mitoses/mm2 (P=0.168). We did not find differences comparing first invasive and subsequent to the first invasive CMs.

Regression

We did not find significant differences in regression comparing the two genotyped groups: the majority of CMs, regardless of mutational status, had no regression (92.7% MUT vs 93% WT of CMs; P=0.944).

Sentinel node metastases (SNMs)

Twenty-seven patients (15.6% among MUT and 22% among WT, P=0.436) met inclusion criteria for sentinel node biopsy (SLNB). None of the 5 MUT patients who underwent this procedure showed metastases (Stage II). Conversely, 18.2% of WTs (4/22) had SNMs (Stage III). However, we did not observe a statistically significant difference in relation to *CDKN2A* gene status (P=0.302).

DISCUSSION

In this paper, we characterized the genetic status of 134 CMs Caucasian patients with respect to the most frequent melanoma susceptibility genes known at present. We found 32 carriers of *CDKN2A* germline mutations (one of which was previously unpublished) and 1 carrier of a unique *CDKN2A* variant; we also identified a carrier of a unique *BAP1* variant previously described by our group [10].

Our study confirms that germline mutation of *CDKN2A* is the main and most frequent genetic susceptibility factor for CM. In contrast, other genes such as *CDK4*, *MITF*, *BAP*1, promoter of *TERT* and *POT1,* which none of the studies published to date and cited in Table 1 has investigated, are probably involved much more rarely.

Herein, we compared the clinical and histopathological features of CMs from 32 carriers of *CDKN2A* germline mutations and 100 non-carriers, demonstrating several significant differences between the two groups.

Our data highlight the overall female predominance in subjects affected by CM. Although the *CDKN2A* germline mutation is transmitted through the autosomal pattern, and the prevalence of the female sex remained constant (ratio F:M approximately 2:1) in both WT and MUT groups. Moreover, most CMs developed in females in both genotyped groups. Caucasian women seem more susceptible to CM, particularly in younger age groups, as confirmed by international European statistics [3,11,16].

Recently, Taylor et al. conducted a multicentre study that included 1928 ethnically different patients. Although most patients, regardless of mutational status, had fair or very fair skin, and the authors observed statistically significant differences between pathogenetic and WT/non-pathogenic *CDKN2A* mutation carriers with respect to sun burning and skin type [36,37]. In contrast with these findings, in our series, we did not find a significant difference in terms of skin type between MUTs and WTs. In our patients, the phototype distribution is comparable to that of the Mediterranean area population, regardless of *CDKN2A* gene status [15]. This finding could depend on the fact that the *CDKN2A* gene encodes two distinct proteins (~~p16INN4~~ p16INK4a and p14ARF) that act as tumour suppressors, while

they have no direct influence on the skin phototype. Nevertheless, it is well known that a light phototype and the presence of *MCR1* variants (RHC, associated with red hair and fair skin) significantly increase penetrance of *CDKN2A* pathogenic mutations [13,27]. Our data are consistent with those published by Aguilera et al., presumably because the two populations share a common ethnic origin (Mediterranean descent) [2].

A high number of melanocytic nevi (>50) and a history of a previous excision of dysplastic nevi, which are identifiable in 7-20% of the European general population, are established risk factors for CM, especially in familial melanoma kindred. Our study reveals that *CDKN2A* carriers, compared to both WT patients to the Caucasian general population, have a higher number of dysplastic nevi. Conversely, the number of common melanocytic nevi is not significantly different compared to WT subjects [20,38,39].

A family history of CM is an important indicator of FCS, in particular of a *CDKN2A* germline mutation. According to the literature data, in our study, the likelihood of detecting *CDKN2A* germline mutation increases with a positive family history of CMs and with the number of family members affected by this cancer [34,35]. The percentage of patients with a positive family history of CMs is significantly higher among MUTs than *CDKN2A*-negative cases (78.1% MUT vs 29% WT, P<0.001). Moreover, the *CDKN2A* gene mutation rate was 40.5% for patients with 2 family members with CM and reached 60% for patients with $\geq$ 3 affected family members. A family history of both CM and PC (9/132) is also associated with a high probability of *CDKN2A* germline mutations (MUT 7/32, 21.9% vs WT 2/100, 2% P<0.001), while a family history of PC alone without family members with CMs (12/132) seems to be less indicative of germline mutations of the *CDKN2A* gene (MUT 4/32, 12.5% vs WT 8/100 8% P=0.441).

It is well known that younger onset of CM is clearly a consequence of *CDKN2A* mutation. In effect, the majority (60.5%) of all CMs in MUTs has been diagnosed by 40 y, and only MUT patients developed CMs before 20 y of age. Nonetheless, because of selection criteria for genetic testing, both MUT and WT index cases showed a higher risk for developing CM earlier (10 to 20 y) than the general population (worldwide mean age: 50 y). Moreover, MUT patients developed CMs without statistically significant anticipation compared to individuals from high-risk melanoma kindred without a mutation (WT). According to the literature, early onset (< 40 y) of the first CM alone without family or personal

history indicative of FCS is not related to a high likelihood of an identifiable mutations on the *CDKN2A* gene [6,34,35,37].

It is known from the literature that *CDKN2A* mutation carriers tend to develop a higher number of MPMs than non-carriers [17,24,27]. This clinical feature is also evident in our experience. MUTs developed a higher number of primary tumours compared both to WT individuals (52% vs 16% P<0.001) and the general Caucasian population (worldwide data 1.3-8.5%) [17]. They also generated skin tumours subsequent to the first with a longer time interval than WTs (data not reported by study summarized in Table 1), despite the fact that we did not find a statistically significant difference.

We also confirmed the well-known difference in the anatomical distribution of CMs between woman and men. Indeed, the body sites most frequently involved in WT female patients were lower limbs and trunk, while WT male patients developed CMs mainly on the trunk (P=0.008, data in accordance with those from the general population) [9]. In contrast, this gender difference disappears if we consider MUT patients (P=0.685). To date, only Aguilera et al. demonstrated differences in tumour localization between *CDKN2A* carriers and control patients, although data on gender differences are missing [2]. Finally, our study provides evidence that CM localization in *CDKN2A* mutation carriers appears to be independent of gender. Moreover, MUTs showed a discordance between the anatomical site of the MPMs in the majority of cases (77.8%) compared to WT patients (47.3%), even though it was not a statistically significant difference (P=0.057). This finding has also been reported by several authors [32,39]. Therefore, we could hypothesize that the anatomical distribution of CMs in carriers of an autosomal dominant *CDKN2A* germline mutation (which confers a constitutional risk to the entire skin surface) might be independent of gender and could be influenced by other factors such as intermittent exposure to UV rays on the trunk and lower limbs. Conversely, the penetrance of the *CDKN2A* mutation (in terms of risk of developing CM) appears to be higher among females, as confirmed previously by other authors (Table 1) [2,25,31,36,39].

Several previous publications have shown that *CDKN2A* carriers tend to develop SSMs and *in situ* CMs more frequently than NMs and LM/LMMs (Table 1). These histological features were also found in our study, without significant differences between genotyped groups (P=0.512), even if we stratified data for specific anatomical sites, except for the head and neck region. Here, there was a prevalence of SSMs in carriers of *CDKN2A* mutations compared to WTs (100% vs 16.7%, P=0.006). Conversely, LM-LMMs (CMs typically related to the oldest patients) were represented only in the WT group. Even

if we consider that these skin tumours were diagnosed in the MUT and WT groups at the same mean age (43 y), these differences could be attributable to the phenotypic characteristics of the genetic syndrome. We also highlighted that MUT subjects developed *in situ* tumours not only more frequently that WT patients (13.4% vs 8.9%) but also earlier, with an average anticipation of 10 years (P=0.105).

Our data confirm the well-known predisposition of mutation carriers to develop invasive CMs with Breslow depths (P=0.023) and Clark levels (P=0.002) significantly lower than WTs, as previously reported by other authors (Table 1). Stratifying data on the basis of the time of diagnosis, differences in terms of Clark level were confirmed for all CMs (first P=0.029 and subsequent to the first CMs P=0.005). With regard to Breslow depth, these differences may be attributable only to invasive CMs (SSMs) diagnosed after the first (SSMs P=0.015). Indeed, the N_CM1 of MUTs showed a Breslow thickness higher than that of WT patients, regardless of histological subtype (although there was no evidence of a statistically significant difference). For NMs, we did not observe a statistically significant difference between the two genotyped groups in terms of Breslow depth (P=0.926) and ulceration (P=0.868). To the best of our knowledge, these findings have never been reported before.

Moreover, among CMs subsequent to the first, *in situ* tumours developed more frequently in WT patients (17.9% vs 42.1%, P=0.068, Table 6). Since all patients, regardless of mutational status, underwent an identical follow-up schedule based on AJCC staging, we hypothesized that this difference might have been determined by the longer mean interval of time between MPMs in MUTs compared to WTs. Specifically, 27.7% of N_CM2 in MUTs (vs 18.75% of WT) was diagnosed $\geq$ 5 y from the first one, when the frequency of physical examinations during the scheduled follow-up decreases. These findings emphasize the crucial role of constant dermatological surveillance, which should be personalized according to mutational status, and it is more important in these high-risk patients to start as early as possible, even for carriers with a negative personal history of CM.

There are few published data on the histological characteristics of CMs developed by *CDKN2A* mutation carriers in terms of tumour ulceration, mitotic rate and regression (Table 1).

For tumour ulceration, in contrast to other data published previously, in our series, the percentage of ulcerated CMs was significantly lower in MUTs than WTs. These differences were significant only in SSMs, whereas we did not find significant differences between NMs developed by MUT and WT patients [31]. In contrast, in our experience, there were no significant differences in mitotic rate or regression comparing invasive CMs from MUTs and WTs. The majority of CMs had mitotic rate <

1/mm^2 and showed no regression, regardless of mutation status. These data were in agreement with those in the literature [2,31].

To our knowledge**,** there are no published data that compare SLNB involvement between MUT and WT patients. In our experience, although the percentage of patients who meet inclusion criteria for this staging procedure were similar in both genotyped groups (P=0.436), there was a greater prevalence of sentinel node metastases (SNM) in WT compared to MUT patients (18.2% vs 0%; P=0.302). These data could be attributable to major prognostic factors, such as the presence of tumour ulceration (17/22, 75% WT vs 0/5, 0% MUT P=0.003) and greater thickness (> 1 mm in 100% WT 22/22 vs 20% 1/5 MUT P<0.001) in WT patients.

In conclusion, our study confirms that *CDKN2A* carriers developed CMs with distinctive features compared to WT/non-pathogenetic *CDKN2A* mutation carriers. They developed *in situ* and invasive CMs with younger onset compared to the general population. Compared to WTs, MUT patients generated *in situ* CMs and metachronous CMs at a lower median age (approximately 10 years earlier).

In comparison to WTs, MUT patients generated a higher number of dysplastic nevi. These data would suggest that melanomagenesis in *CDKN2A* mutation carriers occurs most often by malignant transformation of pre-existing melanocytic precursors (dysplastic melanocytic nevi) rather than from apparently healthy skin (de-novo event).

MUT subjects generated a significantly higher number of MPMs. They also had discordance in the anatomical site and a longer time interval, though we did not find statistically significant differences with respect to WT.

Anatomical distribution of CMs in MUT patients appears to be independent of gender, contrary to WTs. However, female patients seem more susceptible to the development of CM regardless of *CDKN2A* gene status.

MUTs developed SSMs and *in situ* CMs more frequently, while NMs were rare. The number of SSMs on the head/neck region was significantly higher compared to WTs.

For histological findings, MUTs developed SSMs with distinctive characteristics compared to WTs. In detail, SSMs generated by MUT subjects had lower Clark level (for all invasive CMs, regardless of time of diagnosis), lower Breslow thickness (for CMs subsequent to the first) and less frequent ulceration. Conversely, as regard NMs, we did not observe a statistically significant difference between

the two genotyped groups in terms of Breslow thickness and ulceration. NMs developed only as first tumours both in MUT and in WT patients.

CDKN2A carriers rarely developed CMs with regression and high mitotic rate, without statistically significant differences compared to non-carriers.

Since germline mutation of the *CDKN2A* gene segregates independently from skin phototype, we did not find a significant difference in terms of skin type between MUTs and WTs, in contrast to reports by other authors.

COMPLIANCE WITH ETHICAL STANDARDS

Disclosure of potential conflicts of interest: nothing to declare

Funding: nothing to declare

Ethical approval (Research involving Human Participants) and informed consent: this study obtained approval from the Ethics Committee of our Institution (Comitato Etico Interaziendale, Azienda Ospedaliero Universitaria Maggiore della Carità di Novara, Italy). Written informed consent for genetic analysis was obtained from all patients.

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TABLES

We submitted tables as separate “Table”-files

Table 1 - Summary of phenotypic, histopathological and genetics aspects of melanoma *CDKN2A* mutation carriers versus WT melanoma *CDKN2A* patients (data from largest published series).

	Taylor et al.		**Van Der Rhee et al.**			**Sargen et al.**	**Aguilera et al.**		**Masback et al.**	
***CDKN2A* mutation carriers**	670 patients (1258 patients *CDKN2A*-Wild-Type)		182 patients (127 positive and 55 not tested) 7512 control melanoma			123 patients	17 patients		26 patients 78 controls	
Geographical Origin	GenoMel Consortium: Australia, Austria, France, Germany, Italy, Latin America, Latvia, Spain, Sweden, Netherlands, UK, USA		Netherlands			GenoMEL Consortium: USA, Australia, UK, Spain, Italy, Netherlands	Spain		Southern Sweden	
GENDER	Unspecified		M 44% F 56%	**NS** MUT vs control		Unspecified	M 35.3% F 64.7%	**NS**	M 46% F 54%	
SKIN TYPE	Brown/Olive 11% Fair 68% Very fair 21%		**P=0.04**	Unspecified		Unspecified	Fototype I-II: 42.9% Fototype III-IV: 57.1%	**NS** MUT vs WT	Unspecified	
DYSPLASTIC NEVI	Unspecified		Unspecified			Unspecified	Unspecified		Unspecified	
COMMON MELANOCYTIC NEVI	Unspecified		Unspecified			Unspecified	Unspecified		Unspecified	
AGE AT DIAGNOSIS Of CM	<30 y 25% 30-39 y 30% 40-49 y 21% 50-59 y 14% 60-69 y 7% > 70 y 3%	**P<0.0001** MUT vs WT	Mean 39 y	**P<0.001** MUT vs control		Unspecified	Mean 37.51 y	**P<0.05**	Mean 43.5 y	
MPMs	Yes 48% No 52%	**P<0.0001** MUT vs WT	40.7%	**NS** MUT vs control		Unspecified	64.7%	**P<0.005**	Unspecified	
ANATOMIC DISTRIBUTION OF CMs	Unspecified	M Head/Neck 14.4% Trunk 54.3% Upper limbs 13.8% Lower limbs 17.6%	F Head/Neck 10.9% Trunk 29.4% Upper limbs 18,9% Lower limbs 40.8%	**NS** Head/Neck **NS** Trunk **NS** Upper limbs **NS** Lower limbs MUT vs control		Unspecified	Head/Neck 0% Trunk 47% Upper limbs 5.9% Lower limbs 47.1% Palms 0% Soles 0%	**P=0.015** MUT vs WT	Head/Neck 15% Trunk 46% Upper and lower limbs 35% Palms, Soles, and Nails 4%	
DISCORDANCE OF BODY SITE FOR MPMs	Unspecified		63.9%	**P<0.001**		Unspecified	Unspecified		Unspecified	
HISTOLOGICAL SUBTYPE	SSM 73% NM 6% LMM 2%		**P=0.003**	SSM/In situ 88.9% NM 7.6% LMM/LM 2.1%		SSM/In situ OR (95% CI) =1 NM **P<0.001*** LMM/LM **P<0.001***	SSM 91% NM 1.6% LMM 5.7%		In Situ 58.8% Invasive CMs 42,2% SSM 100% Other subtype 0%	
BRESLOW THICKNESS/ INVASIVENESS	In situ:16% 0.01-1.00:62% 1.01-2.00:14% 2.01-4.00: 6% > 4,00: 2%	**P=0.03** MUT vs WT	In situ 22% Invasive 78%	**NS** MUT vs control		Unspecified	Mean 0.74 mm	**P<0.005**	Mean 1.58 mm	**NS**

CLARK LEVEL	Unspecified		Unspecified			Unspecified		Unspecified		II 46% III 31% IV 19% V 4%	**P=0.04**
ULCERATION	Unspecified		Unspecified		Yes 2.4% No 97.6%	**NS** MUT vs WT **NS** MUT vs Sporadic cases **NS** MUT vs WT and Sporadic cases		Unspecified		Yes 27% No 73%	**NS**
MITOTIC RATE	Unspecified		Unspecified		≤1/mm^2 77% >1/mm^2 23%	**NS** MUT vs WT **P<0.0001** MUT vs Sporadic cases **P<0.0001** MUT vs WT and Sporadic cases	≤1/mm^2 13 (76.5%) >1/mm^2 4 (23.5%)	**NS** MUT vs WT	Unspecified	
REGRESSION	Unspecified		Unspecified		No 78% Yes 22%	**NS** MUT vs WT **NS** MUT vs Sporadic cases **NS** MUT vs WT and Sporadic cases	No 88.2% Yes 11.8%	**NS** MUT vs WT	No 69% Yes 31%	**NS**
SENTINEL NODE BIOPSY AND SENTINEL NODE METASTASES	Unspecified		Unspecified		Unspecified		Unspecified		Unspecified	
FAMILY HISTORY OF CM AND PC	Unspecified		Unspecified		Unspecified		Unspecified		Unspecified	
GENETIC ANALYSIS PERFORMED (Susceptibility genes analyzed)	*CDKN2A* *MC1R*	No data about *CDK4*, *MITF*, promoter of *TERT*, *BAP1*,*POT1*	*CDKN2A*	No data about *CDK4*, *MITF*, promoter of *TERT*, *BAP1*, *POT1*, *MC1R*	*CDKN2A*	No data about *CDK4*, *MITF*, promoter of *TERT*, *BAP1*, *POT1*, *MC1R*	*CDKN2A*	No data about *CDK4*, *MITF*, promoter of *TERT*, *BAP1*, *POT1*, *MC1R*	*CDKN2A*	

Notes: only significant values are reported (P<0.05); NS indicates not significant value.

Table 2 - Inclusion criteria to genetic test that strongly suggest a FCS[a] related to melanoma susceptibility genes

Histologically-proven diagnosis of one or more CM and at least one of the following criteria
CM diagnosed ≤ 40 years old
Multiple Primary CMs
Family history of CM
Personal and/or family history of non-cutaneous cancers suggestive of FCS related to germline mutations of *CDKN2A*, *CDK4*, *MITF* and *BAP1* genes

Note a: Familial Cancer Syndrome (FCS) is defined as a hereditable predisposition to a specific pattern of different cancers types and body-sites, aggregating within families, which typically occur as multiple primary malignancies at an earlier age respect the general population.

Table 3 – Phenotypic features of our patients (MUT versus WT) compared to literature data

<table>
<tr><th>CLINICAL FEATURES</th><th>MUT (patients =32 CMs=60)</th><th>WT (patients =100 CMs=119)</th><th>P-Value MUT vs WT</th><th>Previous published data about CDKN2A mutation carriers (Table 1)</th></tr>
<tr>
<td>GENDER</td>
<td>
<table>
<tr><th></th><th>Patients</th><th>CMs</th></tr>
<tr><td>F</td><td>21
65.7%</td><td>43
71.7%</td></tr>
<tr><td>M</td><td>11
34.3%</td><td>17
28.3%</td></tr>
<tr><td>Tot</td><td>32
100%</td><td>60
100%</td></tr>
</table>
</td>
<td>
<table>
<tr><th></th><th>Patients</th><th>CMs</th></tr>
<tr><td>F</td><td>67
67%</td><td>81
68%</td></tr>
<tr><td>M</td><td>33
33%</td><td>38
32%</td></tr>
<tr><td>Tot</td><td>100
100%</td><td>119
100%</td></tr>
</table>
</td>
<td>Patients
M vs F
MUT
P=0.05
WT
P<0.001*

CMs
M vs F
MUT
P=0.005
WT
P<0.001*

Gender
Patients
MUT vs WT
NS

Gender
CMs
MUT vs WT
NS</td>
<td>CONCORDANCE
Predominance of Female regardless genotype</td>
</tr>
<tr>
<td>SKIN PHOTOTYPE
Ethnic origin of our patients: caucasian</td>
<td>
<table>
<tr><th></th><th>Patients</th><th>%</th></tr>
<tr><td>I</td><td>0</td><td>0</td></tr>
<tr><td>II</td><td>8</td><td>25</td></tr>
<tr><td>II</td><td>19</td><td>59,4</td></tr>
<tr><td>IV</td><td>5</td><td>15,6</td></tr>
<tr><td>V</td><td>0</td><td>0</td></tr>
<tr><td>VI</td><td>0</td><td>0</td></tr>
<tr><td>Tot</td><td>32</td><td>100</td></tr>
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</td>
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<table>
<tr><th></th><th>Patients</th><th>%</th></tr>
<tr><td>I</td><td>0</td><td>0</td></tr>
<tr><td>II</td><td>19</td><td>19</td></tr>
<tr><td>II</td><td>65</td><td>65</td></tr>
<tr><td>IV</td><td>16</td><td>16</td></tr>
<tr><td>V</td><td>0</td><td>0</td></tr>
<tr><td>VI</td><td>0</td><td>0</td></tr>
<tr><td>Tot</td><td>100</td><td>100</td></tr>
</table>
</td>
<td>NS</td>
<td>DISCORDANCE
depending on ethnic origin of study population</td>
</tr>
<tr>
<td>DYSPLASTIC NEVI</td>
<td>62.5%</td>
<td>26%</td>
<td>P<0.001</td>
<td>No data
Van Der Rhee cited other references that support high frequency of dysplastic nevi in familial melanoma [39]</td>
</tr>
<tr>
<td>COMMON MELANOCYTIC NEVI</td>
<td>
<table>
<tr><th>N</th><th>Patients</th><th>%</th></tr>
<tr><td><10</td><td>1</td><td>3.1</td></tr>
<tr><td>>10
<50</td><td>20</td><td>62.5</td></tr>
<tr><td>>50</td><td>11</td><td>34.4</td></tr>
<tr><td>Tot</td><td>32</td><td>100</td></tr>
</table>
</td>
<td>
<table>
<tr><th>N</th><th>Patients</th><th>%</th></tr>
<tr><td>< 10</td><td>17</td><td>17</td></tr>
<tr><td>> 10
< 50</td><td>56</td><td>56</td></tr>
<tr><td>> 50</td><td>27</td><td>27</td></tr>
<tr><td>Tot</td><td>100</td><td>100</td></tr>
</table>
</td>
<td>NS</td>
<td>No data</td>
</tr>
</table>

MEDIAN AGE AT DIAGNOSIS OF CMs	**All CMs** 41.5 y (IQR 33.5-49.0 y) *60.5%* ≤ 40 y *39.5%* > 40 y **N_CM1** **First CM** **regardless subtype** 39.5 y (IQR 30.5-45.0 y) **In situ** 34.5 y (IQR 23.0-40.0 y) **N_CM1.5** 29.0 y (IQR 24.0-34.0 y)	**All CMs** 38.0 y (IQR 32.0-45.0 y) *45%* ≤ 40 y *55%* > 40 y **N_CM1** **First CM** **regardless subtype** 37.0 y (IQR 30.5-44.0 y) **In Situ** 43.0 y (IQR 37.5-47.5y) **N_CM1.5** 43.0 y (IQR 39-51 y)	**All CMs** **NS** **N_CM1** **First CM** **regardless subtype** **NS** **In situ** **NS** **N_CM1.5** **NS**	CONCORDANCE Young onset of CM
MULTIPLE PRIMARY CMs (MPMs)	**MPMs** *52%* **Mean CMs per patient** 1.88	**MPMs** *16%* **Mean CMs per patient** 1.18	**P<0.001**	CONCORDANCE Higher number of MPMs
INTERVAL OF TIME (mean, months) BETWEEN THE DIAGNOSIS OF MPMs	**N_CM1 and N_CM2** 53.2 **N_CM2 and N_CM3** 40.5 **N_CM3 and N_CM4** 54	**N_CM1 and N_CM2** 27 **N_CM2 and N_CM3** 21.3 **N_CM3 and N_CM4** --- *none developed 4 CMs*	**N_CM1 and N_CM2** **NS** **N_CM2 and N_CM3** **NS**	**No data**
ANATOMIC DISTRIBUTION of CMs For more details see Table 4	Regardless of gender	Gender differences	MUT (F vs M) **NS** WT (F vs M) **P=0.008**	**CONFLICTING DATA** depending on the case series
DISCORDANCE OF BODY SITE of MPMs	*77.8%*	*47.3%*	**NS**	CONCORDANCE limited data available [39]

Notes: only significant values are reported (P<0.05); NS indicates not significant value.

Table 4 - Anatomical Site of CMs in MUT and WT patients

Anatomical Site of CMs	All MUT 60	All WT 112		MUT F 43	MUT M 17		WT F 74	WT M 38	
Trunk	26 *43.3%* I	54 *48.2%* I	MUT vs WT (regardless of gender) **NS**	17 *39.5%* I	9 *53%* I	MUT M vs F **NS**	31 *41.9%* I	23 *60.5%* I	WT M vs F **P=0.008**
Lower Limbs	16 *26.7%* II	35 31.3% II		12 *28%* II	4 *23.4%* II		31 *41.9%* I	4 *10.5%* III	
Upper Limbs	11 *18.3%* III	17 *15.2%* III		9 *21%* III	2 *11.8%* III		9 *12.2%* II	8 *21.1%* II	
Head and Neck	5 *8.4%* IV	6 *5.3%* IV		3 *7%* IV	2 *11.8%* III		3 *4%* III	3 *7.9%* IV	
Acral sites	2 *3.3%* V	0 *0%* V		2 *4.5%* V	0 *0%* IV		0 *0%* IV	0 *0%* V	

Notes: only significant values are reported (P<0.05); NS indicates not significant value.

Table 5 – Histopathological features of our patients (MUT versus WT) compared to literature data

HISTOPATOLOGICAL FEATURES	MUT (patients =32 CMs=60)	WT (patients =100 CMs=119)	P-Value MUT vs WT	Previous published data about *CDKN2A* mutation carriers (Table 1)
HISTOLOGICAL SUBTYPE§				
SSM	45 *77.6%*	74 *69.1%*	**NS**	CONCORDANCE SSM and In situ are the most frequently histological subtype
In Situ	8 *13.8%*	16 *14.9%*		
NM	3 *5.2%*	13 *12.2%*		
Other	2 *3.4%*	4 *3.8%*		
For more details see Table 6	§ Missing 2/60 *3.3%*	§ Missing 12/119 *10%*		
Concordance of Histological Subtype for MPMs	*63.2%*	*70.8%*	**NS**	**No Data**
BRESLOW THICKNESS				
BRESLOW THICKNESS Median of all invasive CMs	0.4 mm	0.57 mm	**P=0.023**	CONCORDANCE Breslow thickness significantly lower
BRESLOW THICKNESS Mean of all invasive SSM	0.67 mm	0.82 mm	**NS**	**No Data**
BRESLOW THICKNESS Mean of first invasive CMs				
	Regardless subtype 1.04 mm	**Regardless subtype** 0.99 mm	**NS**	**No Data**
	SSMs 0.96 mm	**SSMs** 0.84 mm	**NS**	**No Data**
	NMs 1.73 mm	**NMs** 1.77 mm	**NS**	**No Data**
BRESLOW THICKNESS Mean of invasive CMs subsequent to the first				
	SSMs 0.37 mm	**SSMs** 0.73 mm	**P=0.015**	**No Data**
	NM None	**NM** None	**-**	**No Data**
CLARK LEVEL				
CLARK LEVEL of all invasive CMs§	II 34 *69.4%*	II 38 *39.2%*	**P=0.002**	CONCORDANCE limited data available [25]
	III 14 *28.6%*	III 38 39.2%		
	IV 1 *2%*	IV 20 *20.6%*		
	V 0 *0%*	V 1 *1%*		
	§ Missing 2/51 *3,9%*	§ Missing 3/101 *2.9%*		
CLARK LEVEL of first invasive CMs§	II 14 *53.9%*	II 29 *37.7%*	**P=0.029**	**No Data**
	III 11 *42.3%*	III 32 *41.6%*		
	IV 0 *0.0%*	IV 15 *19.5%*		
	V 1 *3.9%*	V 1 *1.3%*		
	§ Missing 2/28 *7.1%*	§ Missing 2/90 *2.2%*		
CLARK LEVEL of invasive CMs subsequent to the first§	II 20 *87.0%*	II 4 *40.0%*	**P=0.005**	**No Data**
	III 3 *13.0%*	III 3 *30.0%*		
	IV 0 *0.0%*	IV 3 *30.0%*		
	V 0 *0.0%*	V 0 *0.0%*		
	§ Missing 0/23 *0%*	§ Missing 1/11 *9%*		
ULCERATION				

ULCERATION of all invasive CMs§	**Overall CMs** 1.8% **SSMs** 0% **NMs** 33%	**Overall CMs** 11.9% **SSMs** 9,5% **NMs** 38%	**Overall CMs P=0.028 SSMs P=0.036 NMs NS**	**DISCORDANCE** limited data available [25,31]
	§Missing 4/51 *7.8%*	§Missing 16/101 *15.8%*		
ULCERATION of first invasive CMs§	**Overall CMs** 1 4.0% **SSMs** 0 *0%* **NMs** 1 *4%*	**Overall CMs** 10 13.3% **SSMs** 6 *8%* **NMs** 4 *5,3%*	**Overall CMs NS**	**No Data**
	§Missing 3/28 10.7%	*§Missing 15/90 16.6%*		
ULCERATION of invasive CMs subsequent to the first§	**Overall CMs** 0 *0.0%* **SSMs** 0 *0%* **Other subtypes** *0 0%* **NMs** *None of the invasive CMs subsequent to the first was NM*	**Overall CMs** 1 *10.0%* **SSMs** 1 *10%* **NMs and other subtypes** *None (all the of the invasive CMs subsequent to the first were SSMs)*	**Overall CMs NS**	**No Data**
	§Missing 1/23 *4.3%*	§Missing 1/11 *9%*		
MITOTIC RATE < 1/mm²				
MITOTIC RATE < 1/mm² of all invasive CMs	74%	67%	**NS**	CONCORDANCE Majority of CMs with Mitotic rate<1/mm² regardless of mutation status
MITOTIC RATE < 1/mm² of first invasive CMs§	7 *58.3%*	36 *64.3%*	**NS**	**No data**
	§Missing 16/28 *57.1%*	§Missing 35/90 *38.8%*		
MITOTIC RATE < 1/mm² of invasive CMs subsequent to the first§	7 *100%*	7 *100%*	-	**No data**
	§Missing 16/23 *69.5%*	§Missing 4/11 *36.3%*		
REGRESSION				
REGRESSION of all invasive CMs	7.3%	7%	**NS**	CONCORDANCE Majority of CMs with no regression regardless of mutation status
REGRESSION of first invasive CMs§	2 *11.1%*	3 *4.6%*	**NS**	**No data**
	§Missing 10/28 35.7%	§Missing 25/90 *27.7%*		
REGRESSION of invasive CMs subsequent to the first§	0 *0.0%*	1 *14.3%*	**NS**	**No data**
	§Missing 6/23 *26.0%*	§Missing 4/11 *36.3%*		

SENTINEL NODE BIOPSY And SENTINEL NODE METASTASES	**SLNB** 5/32 *15.6%* **SNMs** 0/5 *0%*	**SLNB** 22/100 *22%* **SNMs** 4/22 *18.2%*	**SLNB NS SNMs NS**	**No Data**

Notes: only significant values are reported (P<0.05); NS indicates not significant value; § indicates missing data.

Table 6 - Histological Subtypes of invasive and In situ CMs in relation to mutational status (MUT vs WT)

	ALL INVASIVE CMs 138 49 MUT (+2§) 89 WT (+12§)					**FIRST CMs 119 (+13§) 30 MUT (+ 2§) 89 WT (+11§)**							**CMs subsequent to the first * 47(+1§) 28 MUT 19WT(+1§)**				
	SSM		NM		Other	INVASIVE CMs 26 MUT 79 WT					IN SITU 4 MUT 10 WT		SSM		IN SITU		Other
						SSM		NM		Other							
MUT	45/49 *92%*	**NS**	3/49 *6%*	**NS**	1/49 *2%*	23/26 *88.5%*	**NS**	3/26 *11.5%*	**NS**	0/26 *0%*	4/30 *13.4%*	**NS**	22/28 *78.6%*	**NS**	5/28 *17.9%*	**NS**	1/28 *3.5%*
WT	74/89 *83%*		13/89 *15%*		2/89 *2%*	64/79 *81%*		13/79 *16.5%*		2/79 *2.5%*	10/89 *11.2%*		10/19 *52.6%*		8/19 *42.1%*		1/19 *5.3%*

Notes: only significant values are reported (P<0.05); NS indicates not significant value; § indicates missing data about histological subtypes; *: in this group, we have not detected NMs.

ARCHIVES OF DERMATOLOGICAL RESEARCH THE REVISED MANUSCRIPT (AODR)

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TITLE: MELANOMA PRONE FAMILIES: NEW EVIDENCES OF DISTINCTIVE CLINICAL AND HISTOLOGICAL FEATURES OF MELANOMAS IN *CDKN2A* MUTATION CARRIERS

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Online Resource 1 – Germline mutations and variants in *CDKN2A* genes identified in our study

***CDKN2A* Germline Mutations**	***CDKN2A* Mutation Carriers (Probands)**
c.301G>T (p.Gly101Trp)[a]	24
c.458-2415_471+284del (p.Asp153AlafsX30)[b]	3
c.71G>C (P.Arg24Pro)	3
c.259C>T (p.Arg87Trp)	1
c.142C>A (p.Pro48Thr)	1
c.270C>G (p.Phe90Leu)[c]	1
Tot	33

Note a: The most common worldwide *CDKN2A* mutation identified to date with a particularly high occurrence in France and Italy, due to an ancient founder effect

Note b: *CDKN2A* germline mutation, which was never previously described

Note c: The patient developed three MPMs (diagnosed between 55 and 62 years of age), bilateral ovarian cancer (papillary serous cystoadenoma, at 38 years of age) and invasive ductal carcinoma of the left breast at 59 years of age. The family history was negative for CM, while was positive for endometrial (mother), gastric (maternal grandfather) and ocular (not further specified, maternal uncle) cancers. She carries a *CDKN2A* missense variant, which was never previously described in melanoma pts, while it has been identified as somatic mutation in ovarian and endometrial cancers. Miller at al. published in 2011 a study in which an in vitro functional test showed a significant reduction of the ability of cell cycle arrest in G1 phase (about 30%), like that of the founder germline mutation c.301G>T. [26].

Note d: Carriers' first (FDRs: parents, siblings and children) and second degree (SDRs: grandparents, uncles/aunts, half-siblings, nieces/nephews and grandchildren) relatives who underwent genetic analysis

Note e: Carriers' FDRs and SDRs non-genotyped because they rejected genetic analysis or untraceable or deceased